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ARTICLE

Population PK/PD model of tacrolimus for exploring the relationship between accumulated exposure and quantitative scores in myasthenia gravis patients

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Abstract

Tacrolimus is an important immunosuppressant used in the treatment of myasthenia gravis (MG). However, the population pharmacokinetic (PK) characteristics together with the exposure-response of tacrolimus in the treatment of MG remain largely unknown. In this study, we aimed to develop a population PK/pharmacodynamic (PK/PD) model of tacrolimus in patients with MG, in order to explore the relationships among tacrolimus dose, exposure, and its therapeutic efficacy. The genotype of CYP3A5, Osserman's classification, and status of thymus, as well as demographic characteristics and other biomarkers from laboratory testing were tested as covariate, and simulations were performed based on the final model. The population PK model was described using a one-compartment model with first-order elimination and fixed absorption parameters. CYP3A5 genotype significantly influenced the apparent clearance, and total protein (TP) influenced the apparent volume of distribution as covariates. The quantitative MG scores were characterized by the cumulated area under curve of tacrolimus in a maximum effect function. Osserman's classification was a significant covariate on the initial score of patients with MG. The simulations demonstrated that tacrolimus showed an unsatisfying effect possibly due to insufficient exposure in some patients with MG. A starting dose of 2 mg/d and even higher dose for patients with CYP3A5 *1/*1 and *1/*3 and lower TP level were required for the rapid action of tacrolimus. The population PK/PD model quantitatively described the relationships among tacrolimus dose, exposure, and therapeutic efficacy in patients with MG, which could provide reference for the optimization of tacrolimus dosing regimen at the individual patient level.

Di Chen and Qingyu Yao have contributed equally to this work and share first authorship.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Tacrolimus has been widely used in the treatment of myasthenia gravis (MG), and therapeutic drug monitoring is necessary due to its narrow therapeutic range and high interindividual variability. Thus, several population pharmacokinetic (PK) models have been developed.

WHAT QUESTION DID THIS STUDY ADDRESS?

A population PK/pharmacodynamic (PK/PD) model of tacrolimus in patients with MG was developed to explore the relationships among tacrolimus dose, exposure, and therapeutic efficacy.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Genotype of CYP3A5, total protein (TP), and Osserman's classification were found to significantly influence apparent clearance, volume of distribution, and the initial score of patients with MG as covariates in the population PK/PD model, respectively. A starting dose of 2 mg/d and even higher dose for patients with MG with CYP3A5 *1/*1 and *1/*3 and lower TP level are needed for the rapid action of tacrolimus.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The established model could provide reference for the optimization of tacrolimus dosing regimen in the treatment of MG and the individualized dosing for different subpopulations of patients with MG.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease of neuromuscular junction transmission dysfunction, most commonly caused by antibodies to acetylcholine receptor.[1] MG is associated with characteristic fatigable weakness of the ocular, bulbar, respiratory, axial, and limb muscles. Quantitative MG scores are often used to evaluate the disease status and treatment outcomes in clinical settings.[2]

Immunosuppressants have been considered as effective therapies for MG, including corticosteroids, azathioprine, cyclosporine, cyclophosphamide, and mycophenolate mofetil.[3] In spite of these therapies, tacrolimus, a powerful immunosuppressive agent for treating organ transplantation and autoimmune disease, has emerged as a favorable option in the treatment of MG. In recent years, there has been growing evidence that supports its efficacy and safety in patients with MG, which can lead to a dose reduction of corticosteroids.[4,5] Currently, tacrolimus is often used in the clinical treatment of MG in several countries and is recommended by the international consensus guidance.[6–8]

Tacrolimus has a low oral bioavailability, high protein binding rate (~99%), and strong binding to erythrocyte. It is predominantly metabolized in the liver by CYP3A4 and CYP3A5, and mainly excreted into bile.[9,10] Due to the narrow therapeutic range and high interindividual variability (IIV), individualized dose adjustment based on pharmacokinetics (PKs) is often necessary for the clinical practice of tacrolimus.[11] According to previous literature, several factors, such as pathophysiological status, genetic characteristics, and combined medication, may affect the plasma concentrations of tacrolimus, which contribute to its variability in PKs.[12] Although mostly investigated in organ transplantation, Chen et al. performed a population PK study in Chinese patients with MG where hematocrit (HCT) and blood urea nitrogen (BUN) were identified as covariates that significantly influence the clearance of tacrolimus.[13] Besides, the population PK model developed by Liu et al. demonstrated the effect of CYP3A5*3 genotype and co-administration of Wuzhi capsule on the clearance of tacrolimus in patients with MG.[14] However, these studies have mainly focused on PKs, whereas the relationship between tacrolimus exposure and clinical therapeutic effect (i.e., the quantitative MG scores), remains to be explored.

The aim of this study is to develop a population PK/pharmacodynamic (PD) model of tacrolimus in patients with MG, in order to comprehensively investigate the potential factors that significantly influence the PKs of tacrolimus in patients with MG as well as the

exposure-response relationship, which may further provide some insights into the therapeutic drug monitoring (TDM) of tacrolimus in patients with MG. Moreover, simulations based on the PK/PD model may provide a reference for optimizing the individualized dosage of tacrolimus in patients with MG.

METHODS

Subjects

This study retrospectively analyzed the data of patients with MG receiving tacrolimus treatment in Beijing Hospital from January 2013 to June 2018. Patients aged more than 18 years old with a definitive diagnosis of MG were included in the study. Pregnant women and patients with severe hepatic or renal impairment as well as diagnosis with cancer were excluded. This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Beijing Hospital, and written informed consents were obtained from all study subjects.

Dosage regimen and therapeutic drug monitoring

The initial dose of tacrolimus treatment for patients with MG was 1 mg/d and the dosing interval was 12 h. After taking the same dose of tacrolimus for at least 3 days, the subsequent doses were adjusted according to the clinical efficacy and trough concentration of tacrolimus, of which the target range was 5–10 ng/mL, and the dosing intervals were 8, 12, 24, or 48 h based on the clinicians' decision. Then, 2 mL of venous blood for patients was collected in the anticoagulant tube of ethylenediaminetetraacetic acid (EDTA) 1 h before the next dosing event, and the trough concentrations of tacrolimus were analyzed with chemiluminescent microparticle immunoassay (ARCHITECT I1000SR; Abbott Laboratories, Abbott Park, IL).

Gene polymorphism analysis

Then, 2 mL venous blood of the patients was collected by an EDTA anticoagulation tube at any time for once and total genomic DNA was extracted from peripheral leucocytes. The CYP3A5*36986A>G was detected by digital fluorescence molecule hybridization fluorescence using a fluorescent quantitative polymerase chain reaction instrument and gene analysis system.

Data collection

The demographic information of the patients involved in the study was collected, including sex, age, weight, and height. Besides, the Osserman's clinical classification as well as the thymus status of the patients with MG were recorded. The quantitative MG scores, including blepharoptosis, eyelid fatigue, eye movement, arm fatigue, leg fatigue, facial weakness, chewing, swallowing, and respiratory scores were recorded and added up to the total score (TS), which was considered a quantitative indicator to evaluate the therapeutic efficacy. Laboratory test results were also collected, such as albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, serum creatinine, direct bilirubin, gamma-glutamyltransferase, total protein, serum urea, uric acid, hemoglobin, hematocrit, red blood cells, and white blood cells. In addition, the comedications were collected to investigate their potential interaction with tacrolimus.

Population PK model

One compartment model with first-order elimination rate was selected to describe the PK properties of tacrolimus because only trough data were available in this study. Besides, the absorption rate constant (k_a) was fixed to $0.502\,h^{-1}$ and the absorption time lag was fixed to 0.346 h according to previous literature due to a lack of information about absorption phase in this study.[13] Exponential random effect model was used to describe the IIV:

$$\mathrm{PAR}_i = \mathrm{TVPAR} \cdot \exp(\eta_i) \tag{1}$$

where PAR_i is the parameter estimate for the i_{th} individual, TVPAR represents the population typical value of the parameter, and η_i is the random variable following a normal distribution with a mean of zero and variance of ω.[2]

In addition, additive (Equation 2), proportional (Equation 3), and mix (Equation 4) residual error models were evaluated separately to account for the residual unexplained variability:

$$\mathrm{OBS}_{ij} = \mathrm{IPRED}_{ij} + \varepsilon_{1,ij} \tag{2}$$

$$\mathrm{OBS}_{ij} = \mathrm{IPRED}_{ij} \cdot (1 + \varepsilon_{2,ij}) \tag{3}$$

$$\mathrm{OBS}_{ij} = \mathrm{IPRED}_{ij} \cdot (1 + \varepsilon_{2,ij}) + \varepsilon_{1,ij} \tag{4}$$

where OBS_{ij} is the j_{th} observation of the i_{th} individual, IPRED_{ij} is the j_{th} prediction of the i_{th} individual, $\varepsilon_{1,ij}$ and $\varepsilon_{2,ij}$ represent additive and proportional residual error, following a normal distribution with a mean of zero and variance of

σ_1^2 and σ_2^2, respectively. The base model was selected based on the precision of parameter estimates, objective function value (OFV), goodness-of-fit (GOF) plots, etc.

Covariate effect in PK model

Potential covariates were analyzed following the development of the base model, including the genotype of CYP3A5, comedications, and aforementioned demographic characteristics, as well as laboratory test biomarkers. Patients with CYP3A5 *1/*1 and *1/*3 were mixed as one group due to limited CYP3A5 *1/*1 subjects. In addition, 12 patients with unknown CYP3A5 genotypes were divided into two parts according to the individual apparent clearance (CL/F) estimates in the base PK model (Figure S1), six individuals with higher CL/F estimates were lumped into the CYP3A5 *1/*1 and *1/*3 group, whereas the other six individuals with lower CL/F estimates were lumped into the CYP3A5 *3/*3 group, for the reason that CYP3A5 *1/*1 and *1/*3 were observed at a frequency of 46.7% in Chinese Han, which accounted for nearly half of the population.[15] As for the comedications, pyridostigmine bromide was combined with tacrolimus in the treatment of all the patients, as a result, it would not be involved in covariate analysis. Meanwhile, less than 10% of the patients were combined with atorvastatin, omeprazole, or immunoglobulin, which would also not be included in covariate screening. Stepwise covariate modeling (SCM) was utilized in covariate screening, where the decreased OFV of greater than 3.84 ($p < 0.05$, df = 1) in forward inclusion and the increased OFV of greater than 10.83 ($p < 0.001$, df = 1) in backward elimination of a covariate was considered significant. The effect of continuous covariates was explored with linear, exponential, as well as power model, and shift model was utilized with category covariates. In addition to the statistical significance, biological plausibility and clinical significance were also considered in the covariate model building.[16]

Exposure-response analysis

The PK/PD model was developed to explore the exposure-response relationship of tacrolimus in patients with MG. Empirical Bayes estimates from the final population PK model were used to produce PK profiles, and cumulated area under the curve (cAUC) for each individual was derived to account for the drug exposure during the tacrolimus treatment. The cAUC was calculated by summing up all the AUCs between dosing interval from the beginning of the therapy to the PD assessment.[17] TS obtained from efficacy assessment was treated as a continuous variable in the PK/PD model, and the relationship between TS and cAUC was described by a maximum effect (E_{max}) function:

$$\mathrm{TS} = TS0 \times \left(1 - \frac{E_{\mathrm{MAX}} \times \mathrm{cAUC}}{\mathrm{EcAUC}_{50} + \mathrm{cAUC}}\right) \quad (5)$$

where TS is the estimated total quantitative score of the patients with MG, TS0 is the baseline TS before tacrolimus therapy, E_{MAX} is the max effect of tacrolimus, and EcAUC_{50} is the cAUC required to achieve half of E_{MAX}. Exponential random effect model was used for the IIV of the parameters, and additive, proportional, as well as mixed residual error model were tested for the residual variability.

Covariates were also explored for the PK/PD model. Osserman's clinical classification, status of thymus, comedications, demographic characteristics, and the laboratory test biomarkers were tested on TS0 and EcAUC_{50}, whereas the observed baseline TS was tested on EcAUC_{50}. Patients with unknown thymus status were assumed to have thymic hyperplasia because it has been reported that most of the patients with MG showed abnormal thymus status.[18] Our data also showed that more than 60% of the patients were diagnosed with thymus hyperplasia. The standard for covariate inclusion of the SCM in the PK/PD model was consistent with that in the population PK model, where $p < 0.05$ in forward inclusion and $p < 0.001$ in backward elimination were considered significant. In addition, different functional forms of covariate relationships were also tested, including linear, exponential, and power model for continuous covariates and shift model for category covariates.

Model development and evaluation

The models in this study were developed using first-order conditional estimation with interaction (FOCE-I) in NONMEM (version 7.4.0; ICON Development Solutions) with Perl-speaks-NONMEM (PsN, version 4.9.0). The data profiles and NONMEM outputs were handled with R (version 4.1.2) with package Xpose4 (Uppsala University, Sweden).

The %RSE was used to evaluate the precision of parameter estimates for the final population PK model as well as PK/PD model, and bootstrap (with number of samples, $n = 2000$) was performed to evaluate the robustness of the model and the uncertainty of parameters. GOF plots were utilized to assess the model fits. Besides, normalized prediction distribution errors (NPDEs) and prediction corrected visual predictive check (pcVPC), both based on 1000 simulated datasets, were conducted to evaluate the predictive performance of the final models.

TABLE 1 Summary of characteristics in patients with MG.

Characteristics	Number (%)/ mean ± SD (range)
Age (years)[a,b]	52.4 ± 16.4 (21–87)
Sex (male: female)[a,b]	34 (54.0%): 29 (46.0%)
Weight (kg)[a,b]	69.2 ± 13.4 (40.5–97)
Height (cm)	167.5 ± 7.9 (150–190)
Osserman's classification[b]	
I	7 (11.1%)
IIa	13 (20.6%)
IIb	23 (36.5%)
III	4 (6.3%)
IV	16 (25.4%)
Status of thymus[b]	
Normal thymus	5 (7.9%)
Thymic hyperplasia	42 (66.7%)
Thymoma	7 (11.1%)
Unknown	9 (14.3%)
Comedications	
Pyridostigmine bromide	63 (100%)
Methylprednisolone[a,b]	15 (23.8%)
Atorvastatin	6 (9.5%)
Omeprazole	2 (3.2%)
Immunoglobulin	3 (4.8%)
Amlodipine[a,b]	9 (14.3%)
Nifedipine[a,b]	8 (12.7%)
Genotype of CYP3A5[a]	
*3/*3	28 (44.4%)
*1/*3	16 (25.4%)
*1/*1	7 (11.1%)
Unknown	12 (19.0%)
Laboratory tests	
Albumin (g/L)[a,b]	40.1 ± 3.0 (29.6–52)
Alkaline phosphatase (U/L)[a,b]	68.5 ± 18.0 (39–150)
Alanine aminotransferase (U/L)[a,b]	29.8 ± 24.5 (8–137)
Aspartate aminotransferase (U/L)[a,b]	29.7 ± 12.5 (14–96)
Creatine kinase (U/L)[a,b]	60.1 ± 36.1 (15–189)
Serum creatinine (μM/L)[a,b]	59.9 ± 14.7 (39–121)
Direct bilirubin (μM/L)[a,b]	3.5 ± 1.7 (1–11.5)
Gamma-glutamyltransferase (U/L)[a,b]	28.4 ± 15.9 (9–90)
Total protein (g/L)[a,b]	65.1 ± 15.9 (48–85)
Serum urea (mmol/L)[a,b]	5.1 ± 1.9 (2.5–13.4)
Uric acid (μM/L)[a,b]	287.5 ± 92.9 (136–557)
Hemoglobin (g/L)[a,b]	134.3 ± 13.4 (101–161)
Hematocrit (%)[a,b]	38.8 ± 3.5 (29.7–47)
Red blood cells (10^{12}/L)[a,b]	4.6 ± 0.4 (3.5–5.4)
White blood cells (10^{9}/L)[a,b]	7.2 ± 2.6 (3.1–15.8)

Abbreviations: MG, myasthenia gravis.

[a]Tested in covariate screening of the pharmacokinetic model.

[b]Tested in covariate screening of pharmacokinetic/pharmacodynamic model.

Model applications

In order to assess the benefit for patients from tacrolimus treatment at an early stage of the therapy, the relative total score (RTS) at the end of the second week was utilized to evaluate the therapeutic efficacy, where the patients with RTS greater than 25% could be recognized as responders. RTS could be derived as follows[19,20]:

$$\mathrm{RTS} = \frac{\mathrm{TS0} - \mathrm{TS}}{\mathrm{TS0}} \times 100\,\% \tag{6}$$

where TS is the observed total quantitative score at PD assessment (i.e., at the end of the second week), and TS0 is the baseline TS before tacrolimus therapy. Simulations for different subpopulations were performed to show the impact of covariates on the PK profiles and RTS under various dose regimens based on the final models, where the dosing frequencies were set as twice a day for all dose levels. In addition, a dosing regimen similar to the clinical practice was also simulated. Individuals with different tacrolimus exposure were simulated with different starting daily doses of tacrolimus. After 3 days at a single dose level, the tacrolimus daily doses were increased by 1 mg and finally stopped at a maintaining dose.

Furthermore, the time courses of RTS under 1–6 mg daily dose in subpopulations set with different covariate effect levels were simulated with IIV. One subpopulation contained 1000 virtual individuals with a certain CYP3A5 genotype and TP level.

RESULTS

Patient characteristics

A total number of 107 tacrolimus concentrations were observed in 63 patients, and 255 quantitative MG scores from 55 of them were obtained for analysis. The characteristics are described in Table 1. No patients diagnosed with Osserman's classification V were included in the study, and more than half of the patients were accompanied by thymic hyperplasia. Nearly half of the patients were detected with CYP3A5 *3/*3 alleles, which was similar

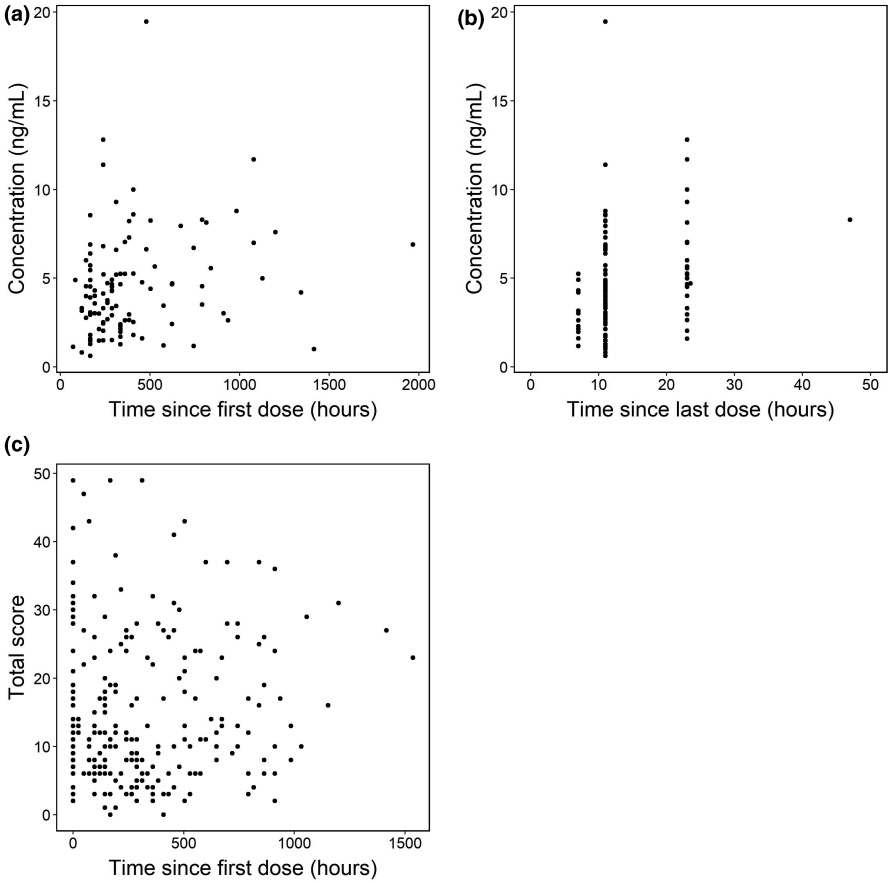

FIGURE 1 Plasma concentration of tacrolimus versus time since first dose (a) and time since last dose (b) as well as the TS of the quantitative MG scores (c). MG myasthenia gravis, TS total score.

to previous literature.[14] The concentration of tacrolimus and the quantitative MG scores used in the population PK model and PK/PD model are shown in Figure 1.

Population PK model

The PK properties of tacrolimus in patients with MG in the current study were described with a one-compartment model with first order absorption rate and a time lag fixed to the values obtained from a previous publication.[13] The parameter estimates of the base model and final model are listed in Table 2, and the GOF plots of the final model are shown in Figure S2.

After the forward inclusion and backward elimination in SCM, the genotype of CYP3A5 was found to be a statistically significant covariate on CL/F, whereas TP significantly influenced the apparent volume of distribution (V/F). The parameter estimates and bootstrap results for the final population PK model are shown in Table 2. The bootstrap results indicated that the model was robust with a minimization success rate of 96.8% from NONMEM, and all the parameters were precisely estimated. The brief summary of covariate screening process is shown in Table S1. The included covariates brought significant decrease in OFV and also reduction in the IIV of CL/F and V/F. The CL/F and V/F of the final model of tacrolimus were described as follows:

$$\mathrm{CL}/F = 15.7, \text{for CYP3A5} * 3/ * 3 \tag{7}$$

$$\mathrm{CL}/F = 15.7 \times 2.05, \text{for CYP3A5} * 1/ * 1 \,\&\, * 1/ * 3 \tag{8}$$

$$\frac{V}{F} = 1410 \times \mathrm{e}^{-0.0817 \times (\mathrm{TP}-65)} \tag{9}$$

where 15.7 is the CL/F for patients with CYP3A5*3/*3 genotype, and the CL/F of patients with CYP3A5*1/*1 and *1/*3 were 2.05-fold higher, 1410 is the typical value of V/F; and 65 is the median of TP. The pcVPC results in Figure S3 indicated that the model had a good predictive performance, whereas the mean value of NPDE showed a slight difference with normal distribution as presented in Figure S4 (p value = 0.0322).

Exposure-response analysis

The PK/PD model describing the relationship between cAUC and TS was developed with an $E_{\max}$ function in Equation 5, where E_{MAX} was fixed to 1, its theoretical maximum value. The parameter estimates are shown in Table 2. The IIV of EcAUC_{50} is 165.8%, indicating a large

TABLE 2 Parameter estimates of the population PK/PD models and the bootstrap results.

	Base model		Final model		Bootstrap results of the final model	
Parameter	**Estimates**	**RSE (%)**	**Estimates**	**RSE (%)**	**Median**	**95% CI**
PK model						
CL/F (L/h)	22.1	7.6	15.7	9.3	15.5	11.9–18.7
V/F (L)	1680	23.0	1410	16.2	1437	1001–2312
k_a (h^{-1})	0.502 FIX		0.502 FIX			
T_{lag} (h)	0.346 FIX		0.346 FIX			
Covariate effect						
CYP3A5*1/*1 and *1/*3 on CL/F			2.05	14.1	2.06	1.52–2.92
TP on V/F			−0.0817	28.9	−0.0766	−0.121–0.00136
IIV CL/F (%)	60.3	11.9	48.3	13.0	47.5	31.7–60.5
IIV V/F (%)	94.9	14.1	74.9	13.9	70.5	29.5–101.1
Residual error						
Proportional error (%)	29.3	13.5	26.8	15.3	26.2	16.9–35.2
PK/PD model						
TS0	11.4	10.8				
TS0 for Osserman I			4.08	27.0	4.02	2.57–7.40
TS0 for Osserman IIa/IIb			10.4	11.5	10.5	8.44–13.2
TS0 for Osserman III/IV			19.0	13.5	19.1	14.5–25.1
E_{max}	1 FIX		1 FIX			
$EcAUC_{50}$ (ng*h/mL)	4090	26.4	4110	33.8	4179	2281–8207
IIV TS0 (%)	74.3	8.5	59.6	8.7	57.8	47.0–67.6
IIV $EcAUC_{50}$ (%)	166.4	13.1	165.8	13.6	165.1	116.4–216.6
Residual error						
Proportional error (%)	24.7	12.0	24.9	12.0	24.7	18.6–30.7

Abbreviations: CI, confidential interval; CL/F, apparent clearance; E_{max}, maximum effect; IIV, interindividual variability; k_a, absorption rate constant; PK/PD, pharmacokinetic/pharmacodynamic; RSE, relative standard error; TP, total protein; V/F, apparent volume of distribution.

variability in patients' response to tacrolimus therapy. Only Osserman's classification was found to be a significant covariate on TS0 (dOFV = −23.293, df = 2, $p < 0.001$), and the TS0 of the final PK/PD model could be described as follow:

$$\text{TS0} = 4.08, \text{for Osserman's classification I} \quad (10)$$

$$\text{TS0} = 10.4, \text{for Osserman's classification IIa/IIb} \quad (11)$$

$$\text{TS0} = 19.0, \text{for Osserman's classification III/IV} \quad (12)$$

where the typical value of baseline TS for patients with Osserman's classification I was 4.08, and patients with classification IIa/IIb as well as III/IV were 10.4 and 19.0, respectively, indicating worse baseline status for those patients compared with the Osserman's classification I subpopulation. The parameter estimates and the bootstrap results for the final PK/PD model are listed in Table 2, showing that all the parameters are precisely estimated. The GOF plots are provided in Figure 2. The NPDE showed no significant difference with normal distribution as presented in Figure S5 (global p value = 0.477), and the pcVPC results in Figure 3 indicated the model had a good predictive performance in 0–500 h, but an underprediction could be found after 500 h.

Model applications

Simulations were performed to visually characterize the PK profiles and RTS for different subpopulations, as shown in Figure 4, where patients with CYP3A5*3/*3 and TP level at 85 g/L (maximum value in the dataset) were defined as high exposure population and patients with CYP3A5*1/*1 and *1/*3 and TP level at 48 g/L (minimal value in the dataset) were defined as low exposure population based on the typical values of the parameters in the PK model. Because TP was a covariate on V/F and individuals with low TP level had much greater V/F compared with individuals with high TP level according to the PK model, the plasma concentration fluctuations in

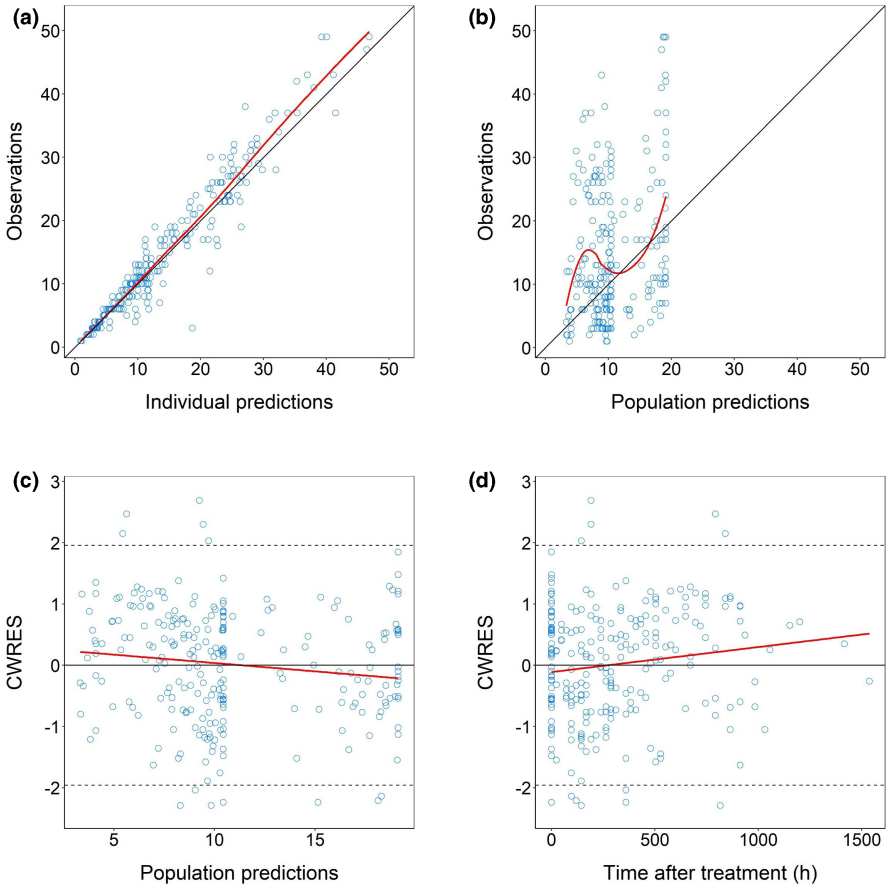

FIGURE 2 GOF plots for the final PK/PD model of tacrolimus in patients with MG, showing observations versus individual predictions (a), observations versus population predictions (b), CWRES versus population predictions (c), and CWRES versus time after treatment (d). CWRES, conditional weighted residual error; GOF, goodness-of-fit; MG, myasthenia gravis; PK/PD, pharmacokinetic/pharmacodynamic.

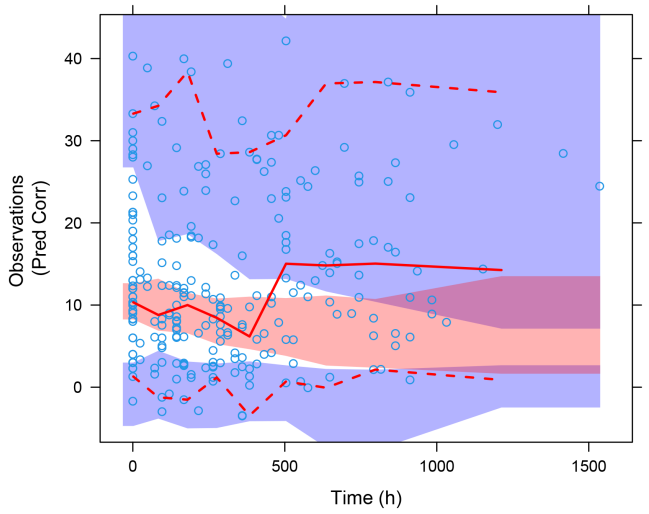

FIGURE 3 PcVPC of the final PK/PD model. The red solid line is the median line of observations, the red shaded area stands for its 90% prediction interval. The red dashed lines are the 5% and 95% percentile of the observations, and the blue shaded area stand for their 90% prediction intervals, respectively. The blue circles refer to observations. Corr, corrected; PcVPC, prediction corrected visual predictive check; PK/PD, pharmacokinetic/pharmacodynamic; Pred. predicted.

the high exposure population were greater than that in the low exposure population. It would take longer time for the population with lower exposure than that with higher exposure under the same daily dose, suggesting that patients with CYP3A5*1/*1 and *1/*3 and lower TP level might require higher dose to respond to tacrolimus therapy at the beginning of the treatment. For the high exposure group, the daily dose of 2–5 mg would be efficacious in 2 weeks even though the steady-state trough concentrations (C_{trough}) for 2 mg was slightly less than 5 ng/mL. As for the low exposure group, the steady-state C_{trough} could not reach the lower limit of 5 ng/mL at a daily dose of 2–3 mg, and the RTS remained below 25% in 2 weeks, indicating that some patients had no rapid response to tacrolimus therapy, possibly due to insufficient exposure.

In the adaptive simulation as shown in Figure 5, it was easy for the individual with high exposure to reach the target plasma concentration range and produced a rapid response to tacrolimus therapy by reaching an RTS of 25% in 2 weeks, when the maintaining dose was no less than 3 mg/d. In addition, the starting dose of 1 mg/d was obviously not enough and at least 2 mg/d was needed. However, individuals with low exposure required higher maintaining dose of 5 mg/d to reach the target TDM range and produce a response within 2–3 weeks. In this situation, a starting dose of 2 mg/d or even higher could be of more value to shorten the time when the patients were exposed to insufficient tacrolimus doses.

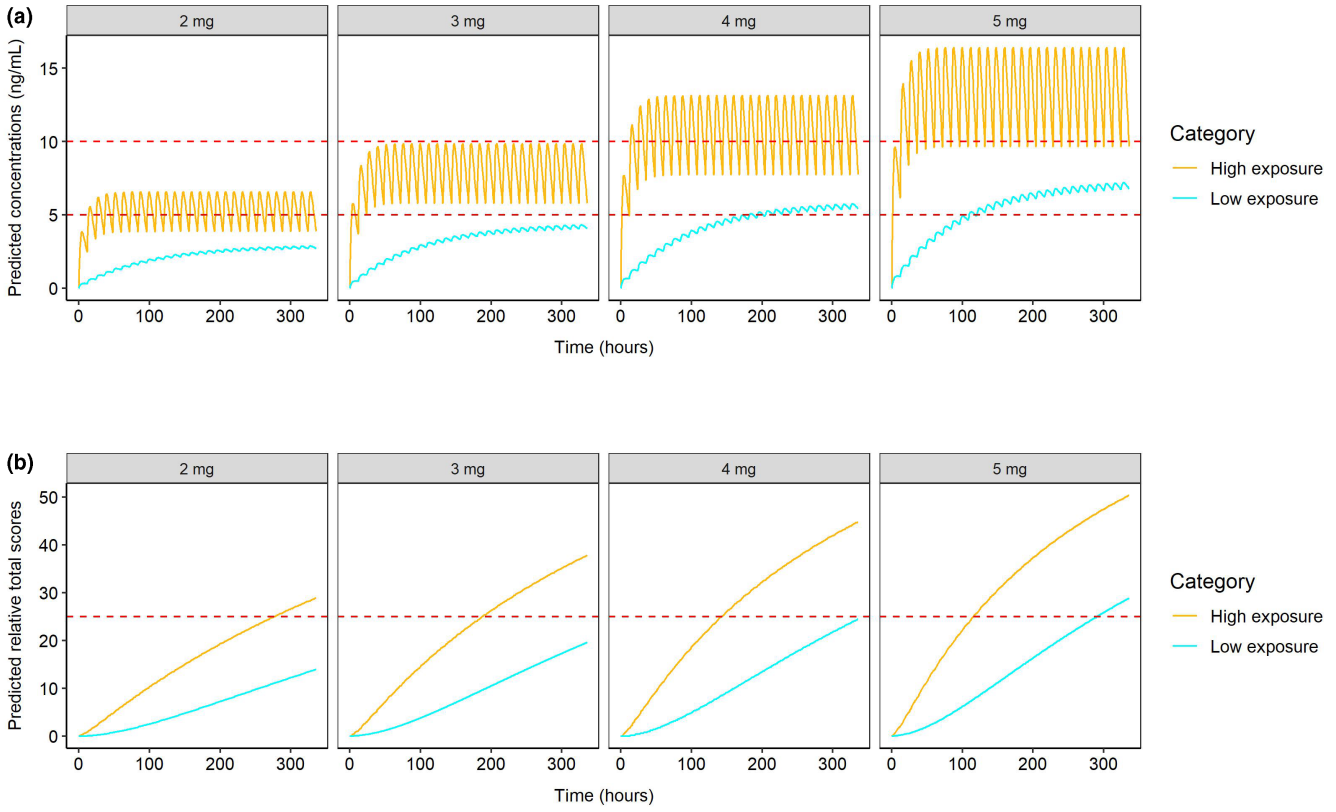

FIGURE 4 Simulations for the time course of tacrolimus concentrations (a) and RTS (b) in the subpopulation with high and low exposure under various daily doses. The dash lines in panel a represent the criteria of 5–10 ng/mL, and the dashed line in panel b stands for the 25% RTS. RTS, relative total score.

In order to further explore the relationship between tacrolimus dose and therapeutic efficacy, the time courses of RTS under 1–6 mg daily dose in different subpopulations were simulated, and 1000 times of simulations were performed for each subpopulation. As illustrated in Figure S6, more than 4 mg of tacrolimus daily dose would be needed for half of the CYP3A5*1/*1 and *1/*3 subpopulation to reach 25% RTS within 2 weeks of treatment, whereas less than 3 mg would be enough for CYP3A5 *3/*3 subpopulation. In addition, with increasing TP levels, more patients could reach 25% RTS within 2 weeks, as Figure S7 showed. Nevertheless, 1 mg of tacrolimus daily dose did not show satisfying efficacy for any subpopulation, which further supported that a starting daily dose of at least 2 mg was more reasonable in the treatment of MG. As for patients with CYP3A5 *1/*1 and *1/*3 as well as lower TP level, even higher doses of tacrolimus might be needed.

DISCUSSION

With a high variability between subjects and narrow therapeutic range, the plasma concentrations of tacrolimus need to be monitored in the clinical treatment of MG.[11] Although there have been two studies focusing on the factors that significantly influence the exposure of tacrolimus in patients with MG using the population PK models,[13,14] the relationship between tacrolimus exposure and the quantitative MG score remains unclear. Another model was developed by Chen et al. in their meta-analysis about drug efficacy evaluation in the treatment of MG, where the drug potencies were estimated and compared among therapeutic antibodies and immunosuppressants, including tacrolimus, but the relationship between exposure and response was less considered.[21] In this study, the population PK model and PK/PD model of tacrolimus in patients with MG were sequentially developed, in order to provide reference for tacrolimus dose selection not only based on C_{trough} but also quantitative MG scores, which was more directly connected with the therapeutic efficacy.

Because the data were obtained from the clinical treatment of patients with MG and only C_{trough} of tacrolimus were recorded, the parameters associated with absorption phase could not be well-estimated due to limited information. Thus, the k_a and T_{lag} were fixed to the values estimated from healthy volunteers.[13] Another study performed on pediatric and adolescent kidney transplant recipients provided different k_a estimate of 0.75 h^{-1}, which was significantly different from the population of this study in

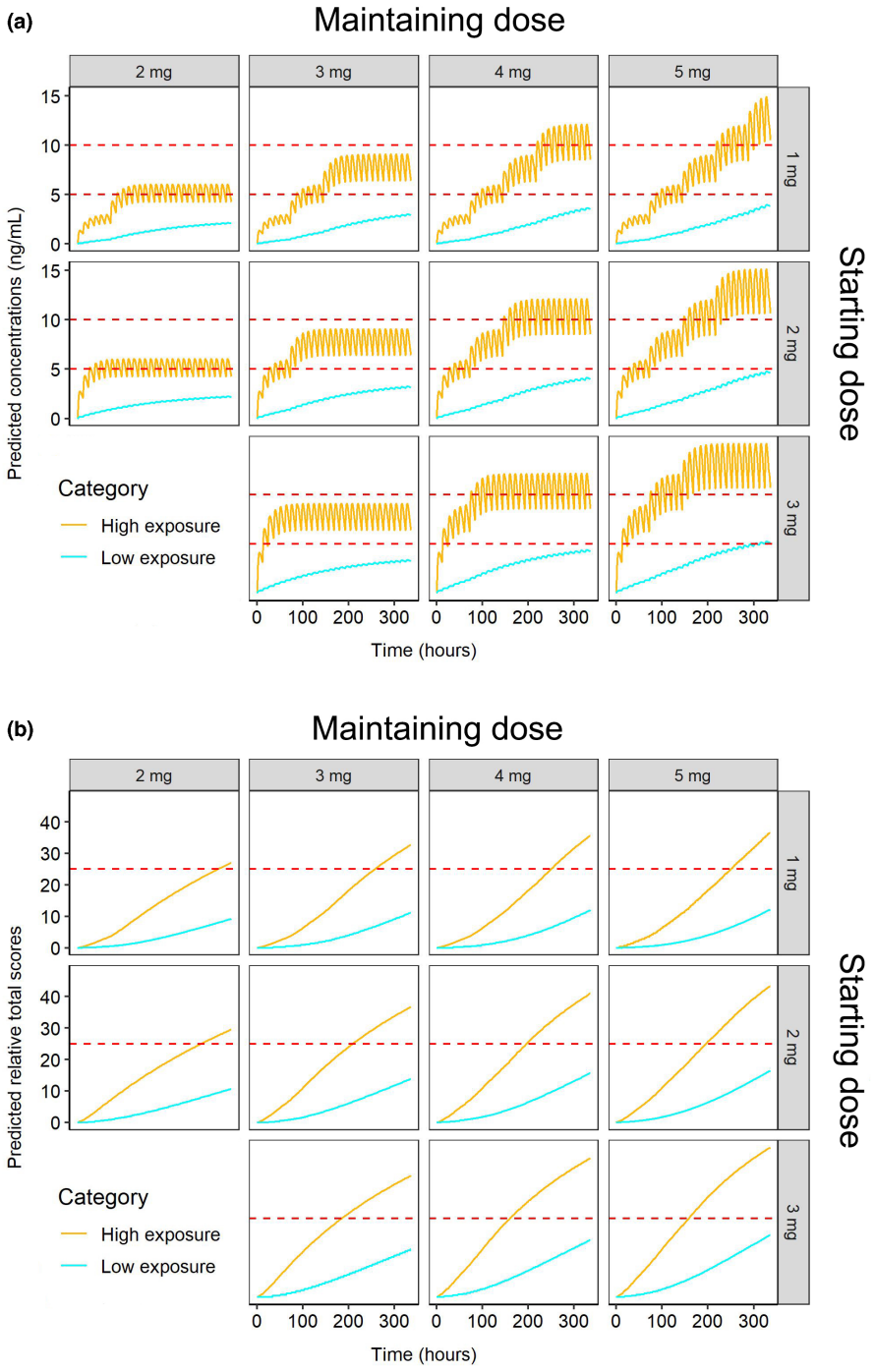

FIGURE 5 Simulations for the time course of tacrolimus concentrations (a) and RTS (b) in the subpopulation with high and low exposure under adaptive dosing regimen. After 3 days at a single dose level, the tacrolimus daily doses were increased by 1 mg and finally stopped at a maintaining dose. The dash lines in panel a represent the criteria of 5–10 ng/mL, whereas the dashed line in panel b stands for the 25% RTS. RTS, relative total score.

demographic characteristics (age from 21 to 87 years) as well as in disease, and therefore it was not adopted in this study.[22] In addition, two-compartment models were used in other studies for the population PK model of tacrolimus.[23–25] We have also tested a two-compartment model in this study but it did not successfully converge, and the volume of distribution of the periphery compartment was close to zero, suggesting that the model tended to collapse to a one-compartment model. Because the sparse data in this study did not provide sufficient information for developing a two-compartment model, a one-compartment model could be more adequate and fit-for-purpose.

There was missing covariate information in the datasets on CYP3A5 and thymus status. Because the CYP3A5 *1/*1 and *1/*3 genotypes were accounted for nearly half of the population in this study,[15] and it was also well studied that CYP3A5 *3/*3 could result in lower clearance,[10,14] the 12 patients with unknown CYP3A5 genotype were therefore divided according to CL/F estimates, the higher half were lumped into the CYP3A5 *1/*1 and *1/*3 group, whereas the lower half were lumped into the CYP3A5 *3/*3 group, which might lead to a lower bias compared with lumping the 12 individuals into either one group and could be more reasonable. Besides, the nine patients

with unknown thymus status were assumed to have thymus hyperplasia, which accounted for the majority of the population.[15,18] Although it may bring some bias to the parameter estimation, it would be better to make these assumptions rather than just exclude these individuals from the datasets, considering the limited small sample size in this study.

The genotype of CYP3A5 has been widely reported to be a significant covariate on the clearance of tacrolimus either in MG or organ transplantation recipients, in which CYP3A5 *1/*1 has the highest clearance, whereas CYP3A5 *3/*3 has the lowest.[12,14] However, due to limited CYP3A5 *1/*1 subjects in the dataset, the parameter for covariate effect of CYP3A5 *1/*1 on clearance could not be precisely estimated (RSE > 100%). Therefore, these individuals were finally mixed with the CYP3A5 *1/*3 subpopulation, so as to stabilize the model. CYP3A4 *22 also showed strong effect on the CL of tacrolimus in White patients,[24,26] but it was not detected in this study because it is basically absent in east Asia according to the literature.[27,28] Besides, TP was included as a covariate on CL in the model developed by Golubovic et al. where the volume of distribution was fixed.[29] In this study, TP was found to influence the V/F as a significant covariate, which may attribute to the high protein binding rate of tacrolimus.[9] Other potential covariates, such as HCT, BUN, age, and body weight were also tested in this study.[13,30] However, none of them were included after the SCM analysis.

Some misspecifications in the PK model could be found according to the GOF plots in Figure S2, which might be attributed to the non-included covariates. However, the acceptance criteria of covariate effect were not set as $p < 0.01$ due to the limited data, in order to keep covariates with more significance in the model and also lower the risk of overparameterization. Some trends of NPDE could be seen after 800 h in plot C as well as under 2 ng/mL or over 6 ng/mL in plot D of Figure S4, possibly due to relatively sparse data in these intervals. In addition, comedications, such as Wuzhi capsules, might influence the exposure of tacrolimus.[14] In the current study, atorvastatin, omeprazole, as well as immunoglobulin were not analyzed due to small sample size, and other comedications were not included in the final population PK model. More data are required for further analysis of comedications.

Following the established population PK model, various attempts have been made to quantitatively describe the relationship between tacrolimus exposure and therapeutic efficacy. At first, PK/PD models using the time course of tacrolimus plasma concentration were tried with direct response, indirect response, as well as biophase model on the MG scores.[31] However, none of these models provided good fits and reasonable results. Then, maximum plasma concentration, AUC, and cAUC were tested for the model instead of time-varying concentration,[17,32] and cAUC was finally selected as a better predictor for the current dataset, which assumed that the quantitative MG scores were directly associated with cumulative tacrolimus exposure. According to previous literature, tacrolimus might manifest on or after 3 months of therapy.[33] However, the longest PD observation happened on day 64, leading to a relatively low effect of tacrolimus on TS due to the relatively short time, which is a limitation of the study. In addition, according to our attempts, the model could not estimate E_{MAX} and its IIV as well as the IIV of $EcAUC_{50}$ at the same time, due to the limitation of the dataset. As a result, E_{MAX} was fixed to 1 in the model, which was the theoretical maximum for the parameter and also actually achieved by some patients in this study.

In the covariate analysis of the PK/PD model, Osserman's classification was found to be significantly related to the TS0, which was comprehensible because the Osserman's classification was decided by clinical symptoms and disease progression, and the severity increased from classification I to V.[34] However, it would be difficult to estimate all those five classifications during modeling, given that only three individuals were involved in Osserman's classification III in the dataset for the PK/PD model. Thus, in this study, Osserman's classifications were re-organized into three groups according to the clinical symptoms as well as sample size, in order to obtain a more robust model. In addition, the status of thymus as well as age were also found to be related to the morbidity and progression of MG,[1,35] and significant OFV reductions were also found in the first round of forward inclusion of covariate on TS0 (dOFV = −6.956 for thymus, and dOFV = −6.019 for age), but none of them were reserved in the final model due to the criteria of backward elimination ($p < 0.001$), as shown in Table S2. Besides, no covariate was found on the drug-related parameter $EcAUC_{50}$. It might require more information to quantify the interaction between tacrolimus and the comedications, either from the PK or PD aspect.

Some misspecifications could be seen in the GOF plot of the PK/PD model (Figure 2), where the population predictions were underestimated for high scores, and the conditional weighted residual tended to increase after 500 h. Besides, the pcVPC indicated that the model showed good predictive performance in the first 500 h of tacrolimus treatment, but the median of the observations was obviously higher than the predictive interval after 500 h. On the one hand, the problems might come from the varying observation duration of different individuals. Patients with severer MG (i.e., higher TS) generally received longer treatment, thus generating more observation data in the dataset, especially after 500 h. In

addition, the observation data were not well-distributed, where 193 observations (75.7% of the total number) were within 0–500 h, whereas only 62 observations (24.3%) occurred between 500 and 1536 h. On the other hand, the misspecification could also be due to the assumption that E_{max} was fixed to the theoretical maximum 1, where some patients did not reach this maximum due to the relatively short treatment time in this study. These limitations indicated that the current model may not be so reliable in predicting a long-term efficacy of tacrolimus therapy of MG, and more data with longer observation duration would be required in future studies. Nevertheless, because the simulations in this study were limited to the first 2 weeks, and the conclusions were mainly focus on the starting dose of tacrolimus, the current model was still considered fit-for-purpose and could be useful in spite of some misspecifications.

The target criteria of trough concentration were set as 5–10 ng/mL in the TDM of tacrolimus based on the published literature and experience from clinical practice,[14,36] which was higher than the 2–9 ng/mL in the Chinese guidance of MG therapy.[37] According to the simulations, tacrolimus had fast response within 2 weeks when the lower limit of 5 ng/mL C_{trough} in steady-state was reached, suggesting that higher tacrolimus TDM criteria could bring faster therapeutic effect on the patients. More safety data are needed to derive the upper limit of tacrolimus TDM criteria, which was not involved in the current study. The starting dose of tacrolimus treatment could be 2 mg/d and even higher for patients with CYP3A5 *1/*1 and *1/*3 as well as lower TP level based on the simulations, which was in accordance with previous reports where the CYP3A5 genotype influenced the starting dose of tacrolimus.[38,39] A limitation of the model is that the RTS derived by Equation 6 could be directly dependent on cAUC in Equation 5, and, therefore, RTS is proportional to cAUC, which is not completely reasonable due to the large variability in patients with MG. Because the Osserman's classification was added as a covariate on TS0, which was the initial quantitative MG score and not related to the therapeutic effect of tacrolimus, patients with different Osserman's classifications were not simulated due to the limitation of the PK/PD model.

A limitation of this study is that the datasets used for developing the population PK model contained only C_{trough} of tacrolimus, without the necessary information to estimate absorption parameters. More informative data and larger sample size could improve the model performance in future studies. Another limitation is that placebo data were not included in this study. Because the quantitative MG score was not an objective clinical evaluation index, a placebo model would be helpful to separate the placebo response from the therapeutic effect. Besides, the daily dose of the patients was adjusted according to the TDM target criteria rather than fixed dosage regimen. Therefore, the exposure-response relationship might also be limited, extrapolations for higher tacrolimus doses, as well as longer treatment time should be interpreted with caution. Nevertheless, the population PK/PD model developed in the current study could quantitatively describe the relationship between tacrolimus exposure and quantitative MG scores, which fitted the purpose for the optimization of tacrolimus dosing regimen.

In summary, a population PK/PD model was developed to quantitatively describe the relationships among the dose, exposure, and therapeutic efficacy of tacrolimus in the treatment of patients with MG. The starting dose of tacrolimus could be 2 mg/d and even higher for patients with CYP3A5 *1/*1 and *1/*3 and lower TP level based on the simulations. The established model could provide reference for the optimization of tacrolimus dosing regimen in the treatment of MG as well as the individualized dosing for different subpopulations of patients with MG.

AUTHOR CONTRIBUTIONS

D.C., Q.Y., T.Z., and P.J. wrote the manuscript. D.C., Q.Y., T.Z., and P.J. designed the research. D.C., J.Y., S.H., X.T., M.Z., H.Z., and L.Y. performed the research. Q.Y. and W.C. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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